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Original article

Clinical phenotypes associated with circulating tumor cell enumeration in metastatic castration–resistant prostate cancer

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Abstract

Background: The presence of ≥5 circulating tumor cells (CTCs) is prognostic for shorter survival in men with metastatic castration–resistant prostate cancer (mCRPC). However, some men have low CTCs despite widespread disease, suggesting heterogeneity in CTC phenotype or detection. The aim of this study was to evaluate the association of CTC enumeration with clinical disease characteristics and overall survival in men with mCRPC at our institution.

Design: CTCs were enumerated using the CellSearch method in a prospective correlative study in men with mCRPC starting a new systemic therapy. The primary objective was to determine the clinical phenotype of the subset of men with mCRPC who have a poor prognosis and low CTCs. Secondary end points included associations of CTCs with survival and known prognostic biomarkers, before therapy and at progression.

Results: At baseline, median CTC count was 16 cells and prostate-specific antigen (PSA) level was 178 ng/ml. At progression, median CTC count was 42, PSA level was 245 ng/ml, levels of lactate dehydrogenase and alkaline phosphatase rose, and level of hemoglobin dropped. The median overall survival for this heavily pretreated population was 11.2 months, and the multivariable hazard ratio for death of men with CTCs <5 vs. ≥5 was 0.43 (95% CI: 0.24–0.77). Median progression-free survival was 4.4 months. CTC enumeration modestly correlated with lactate dehydrogenase and alkaline phosphatase levels but only weakly correlated with PSA and hemoglobin levels. We were unable to identify a consistent subgroup of poor prognosis men with a low number of CTCs.

Conclusion: CTC enumeration appears to be prognostic in men with mCRPC and describes a phenotype of hematogenous dissemination that cannot be predicted based on standard clinical and laboratory assessments.

Keywords: Circulating tumor cells; Prostate cancer; Tumor biomarkers; Prognosis; Prostate specific antigen

1. Background

Circulating tumor cells (CTCs) are cells that have migrated from primary or metastatic tumor sites and intravasated into the circulation. The presence of ≥5 CTCs using the CellSearch epithelial cell adhesion molecule (EpCAM)–based ferromagnetic assay is prognostic for shorter survival in men with metastatic castration–resistant prostate cancer (mCRPC) [1], and CTCs can be enumerated to provide prognostic information in multiple other solid tumor types [2–5]. However, a substantial number (30%–40%) of men with advanced CRPC have low or undetectable CTCs using the CellSearch epithelial-based method, despite widespread metastatic disease [6]. Although these men with low CTCs, despite having progressive disease, have an improved prognosis compared with those with higher CTCs, they represent a heterogeneous group, and

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outcomes remain poor with median survival estimates of only 1.5 to 3 years.

In women with metastatic breast cancer, patients with aggressive disease phenotypes such as triple-negative histology, inflammatory breast cancer, or brain metastases have fewer detectable CTCs than expected for their burden of disease, in spite of their poor prognosis [7]. This underdetection of CTCs may be owing to phenotypic plasticity, rendering epithelial-based CTC capture less useful [8,9]. Several intrinsic phenotypes of breast cancer lack EpCAM expression, and cells from these tumors go undetected using the standard EpCAM-based assay [10]. In many other aggressive metastatic solid tumors such as lung cancer or gastrointestinal cancers, the underdetection problem may be even more manifest [4,11,12].

There is evidence for phenotypic heterogeneity among CTCs, with some CTCs expressing not only epithelial proteins, but also mesenchymal and stemness proteins, indicators of epithelial plasticity (EP) [8]. EP refers to the reversible loss of the epithelial cellular phenotype and has been linked to the acquisition of mesenchymal or stemness properties and to chemoresistance, invasion, and dissemination in multiple preclinical models of cancer [13,14]. In prostate cancer, mesenchymal biomarkers are up-regulated during androgen deprivation therapy, revert upon replacement of testosterone, and are linked to an increased metastatic propensity and chemotherapy resistance [15–17]. Recent studies have suggested links between EP and resistance and progression despite radical prostatectomy, radiation, hormonal therapy, and immunotherapy (reviewed in Ref. [18]).

Although there are many methods under development to capture CTCs from the blood of patients with cancer, the only Food and Drug Administration–cleared technology is CellSearch, which uses an anti-EpCAM ferrofluid to capture CTCs and follows with additional staining to visualize the cells and differentiate them from leukocytes. Using CellSearch, CTCs have been shown to be extremely rare in individuals without malignancy and present at a wide range of frequencies in patients with various metastatic carcinomas [11]. Owing to EP, CTCs with a mesenchymal or transitional phenotype may be missed by CellSearch and other epithelial-based technologies [10,19,20].

A range of clinical phenotypes exist in men with castration-resistant metastatic prostate cancer (reviewed in Ref. [21]). These phenotypes are determined by the pattern of metastatic spread (visceral, bone, and node only), prostate-specific antigen (PSA) production, anemia, symptoms such as pain and fatigue, levels of bone biomarkers such as alkaline phosphatase (AP) level, and histology such as adenocarcinoma and neuroendocrine variant tumors. These clinical phenotypes are linked to survival and are commonly used to determine prognosis in the clinic and eligibility/stratification for clinical trials. Several studies have examined the association of clinical phenotype with CTC enumeration, illustrating that CTCs are more common in men with bone metastases and high PSA level, and in the postdocetaxel CRPC setting [1,22]. These studies have established the independent association of CTC enumeration with survival [1,3,23]. However, the systematic examination of the association of clinical phenotype in men with a low vs. high CTC count has not been performed.

Here we evaluated the association of CTC enumeration, both before a new systemic therapy and at progression on a given systemic therapy, with baseline characteristics and clinical outcomes in men with mCRPC at the Duke Cancer Institute. We hypothesized that men with mCRPC and Gleason score 8 to 10 disease, visceral metastases, low PSA production, or pain would have shorter survival duration but lower CTCs than expected, similar to that reported in patients with triple-negative breast cancer [7]. We speculated that poorly differentiated tumors may have lost their epithelial character, at least in part, and may have CTCs that lack EpCAM expression and are thus underdetected by the CellSearch assay despite a poor prognosis.

2. Methods

Men with progressive, mCRPC consented and were enrolled in 1 of 2 institutional review board-approved prospective correlative clinical protocols before initiating a new systemic therapy. Eligibility for this study included the presence of metastatic disease, progression by PSA, bone scan, or soft tissue/visceral disease criteria (new lesions or progression of existing lesions), and a serum testosterone level of <50 ng/dl. Men were enrolled before initiating a new systemic therapy for CRPC, including enzalutamide, docetaxel, abiraterone acetate, cabazitaxel, or novel agents on a separate clinical protocol. Men were excluded if they had received an anthracycline or mitoxantrone within 7 days of blood draw to reduce the risk CTC autofluorescence caused by these agents. Per protocol, men underwent CTC collection at baseline, after 3 months on treatment, and at progression. All other laboratory and imaging tests were performed per the discretion of the treating physician, at least every 3 months. All the men provided informed consent.

The EpCAM-based CellSearch platform was used for CTC detection and enumeration, as described previously [11]. The results of cell enumeration were expressed as the number of cells per 7.5 ml of blood. CTC enumeration was performed at baseline in all men before the initiation of a new treatment. Men also had blood drawn for CTC enumeration following progression as determined by the treating physician (clinical, radiographic, or PSA progression). Laboratory studies collected and measured as part of the standard-of-care included baseline hemoglobin (Hgb) level, lactate dehydrogenase (LDH) level, AP level, liver and kidney function, and PSA level. Clinical parameters analyzed included pattern of metastatic spread (the liver, lung, bone, and lymph node metastasis patterns), pain on a